

SVCL
P

FIELD OF INVENTION

2 This invention relates to neuroprobes for mapping monoamine
3 reuptake sites in the brain, and particularly to a neuroprobe that
4 can also serve as a radiotracer for use in single-photon emission
5 computed tomography (SPECT) and positron emission tomography (PET)
6 for imaging of such reuptake sites.

CL
P

BACKGROUND OF THE INVENTION

8 A brain consists of a plurality of neurons that interact by
9 exchanging chemical messengers. Each neuron generates
10 neurochemicals, referred to as neurotransmitters; neurotransmitters
11 act at sites on the cellular membrane of a neuron, the sites being
12 referred to as receptors. Receptors are associated with either ion
13 channels through the cellular membrane or secondary neurochemical
14 messenger systems. By contrast, reuptake sites are molecular
15 complexes which transport chemicals across the cellular membrane of
16 a neuron. When a neurotransmitter has served its function, it is
17 removed from the vicinity of the receptor by being bound to a
18 reuptake site which transports the neurotransmitter to the interior
19 of the neuron.

20 Just as there are many specialized neurons in the brain, there
21 are also a variety of neurotransmitters, associated receptors, and
22 reuptake sites. The distribution of specialized neurons depends
23 upon the particular organism under study, and the state of health
24 of that organism.

1 A neuron can be classified according to the type of
2 neurotransmitter that it uses to communicate with other neurons.
3 Certain types of neurons can be found predominantly in particular
4 regions of the brain. For example, the striatal region of a
5 mammalian brain is innervated by neurons using dopamine as a
6 neurotransmitter. The striatum also contains a large number of
7 non-dopaminergic neurons that have dopamine receptors. Certain
8 compounds, such as cocaine, have a preferential affinity for
9 dopamine reuptake sites, and therefore tend to bind to such
10 reuptake sites. The effect of a molecule such as cocaine upon a
11 dopamine reuptake site is to inhibit reuptake of the
12 neurotransmitter dopamine, leaving more dopamine available in the
13 vicinity of the dopamine receptors.

14 In certain neurological diseases, such as Parkinson's disease,
15 distinct groups of neurons lose their normal physiological
16 functioning. Consequently, the abnormal neurons may behave
17 differently in the presence of some neurotransmitters, and may also
18 produce neurotransmitters in a manner that differs from a healthy
19 neuron.

20 The major neurotransmitters, dopamine, norepinephrine, and
21 serotonin, are referred to collectively as the monoamine
22 neurotransmitters. Many neurons have receptors adapted to receive
23 at least one of these neurotransmitters. Parkinson's disease is
24 caused by the degeneration of some of the dopaminergic neurons in
25 the brain. The neurons lost in Parkinson's disease have a large

1 number of dopamine reuptake sites; cocaine and chemical analogs of
2 cocaine have an affinity for such reuptake sites.

3 A radioisotope is commonly incorporated in molecules that have
4 a demonstrated binding affinity for a particular type of
5 neuroreceptor, and such molecules are commonly used as neuroprobes.
6 The localization of neuroprobes can be used to find specialized
7 neurons within particular regions of the brain. It is also known
8 that a neurological disease can be detected by observing abnormal
9 binding distributions of a neuroprobe. Such abnormal binding
10 distributions can be observed by incorporating a radionuclide
11 within each molecule of the neuroprobe with a high binding affinity
12 for the particular reuptake sites of interest. Then, an imaging
13 technique can be used to obtain a representation of the in vivo
14 spatial distribution of the reuptake sites of interest.

15 In single photon emission computed tomography (SPECT) imaging,
16 the most commonly used radionuclides are heavy metals, such as
H 17 ^{99m}Tc. Heavy metals are very difficult to incorporate into the
18 molecular structure of neuroprobes because such probes are
B 19 relatively small molecules (molecular weight less than 400).

20 In positron emission tomography (PET), the radiohalide ¹⁸F
21 (fluorine) is commonly used as a substitute for H (hydrogen) in
22 radiopharmaceuticals because it is similar in size. Not all
23 halogens will work, however. For example, I (iodine) is much
24 larger than both H and F, being approximately half the size of a
25 benzene ring. However, due to the small size of typical
26 radiopharmaceuticals for use as neuroprobes, the presence of iodine

1 markedly changes the size of the compound, thereby altering or
2 destroying its biological activity.

3 In addition, the presence of iodine in a neuroprobe tends to
4 increase its lipophilicity, and therefore increases the tendency of
5 the neuroprobe to engage in non-specific binding. For example,
6 paroxetine is a drug with high affinity and selectivity for
H 7 serotonin reuptake sites, and [³H]paroxetine has been shown in
8 rodents to be a useful in vivo label (Scheffel, U. and Hartig, PR.
B14 9 J. Neurochem., 52: 1605-1612, 1989). However, several iodinated
10 analogs of this compound with iodine attached at several different
11 positions had unacceptably low affinity, in fact being one tenth of
12 the affinity of the parent compound. Furthermore, when the
13 iodinated compound was used as an in vivo radiolabeled neuroprobe,
14 non-specific binding activity was found to be so high that no
15 measurable portion of the brain uptake appeared to be specifically
16 bound to the serotonin reuptake site. Thus, the iodinated form of
17 paroxetine is not useful as an in vivo probe.

18 The addition of iodine to a neuroprobe can unfavorably alter
19 its biological properties. For example, tomoxetine has high
20 affinity and selectivity for norepinephrine reuptake sites.
21 However, when tomoxetine is iodinated, e.g. to form R-4-
22 iodotomoxetine, the resulting labeled compound has low affinity for
23 such reuptake sites, and relatively high affinity for serotonin
24 reuptake sites. In vivo labeling studies have shown that it is an
25 unacceptably poor probe even for the serotonin reuptake sites

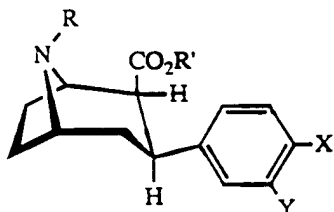
1 because it exhibits low total brain uptake and immeasurably low
2 specific uptake.

3 An iodinated compound can be useful as an in vitro probe, but
4 may be useless as an in vivo probe, because an in vivo probe must
5 meet the requirements associated with intravenous administration of
6 the probe to a living subject. Reasons for the loss of in vivo
7 utility include the fact that the compound may be metabolized too
8 quickly, that it may not cross the blood-brain-barrier, and that it
9 may have high non-specific uptake into the lipid stores of the
10 brain. In vitro homogenate binding studies remove these obstacles
11 by isolating the brain tissue from hepatic metabolic enzymes, by
12 homogenizing the brain tissue so as to destroy the blood-brain-
13 barrier, and by diluting the brain tissue so as to decrease the
14 concentration of lipids in the assay tube. Accordingly, it cannot
15 be assumed that a probe will be useful in both in vivo and in vitro
16 modalities.

17 An in vivo SPECT probe was developed by iodinating cocaine.
18 However, this probe shows a binding affinity and specificity no
19 better than cocaine itself, which is inadequate for purposes of
20 SPECT imaging.

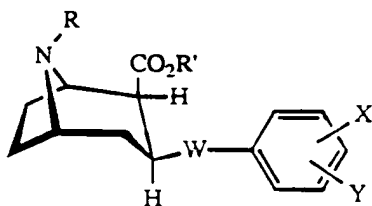
SUMMARY OF THE INVENTION

An iodinated neuroprobe is provided for mapping monoamine reuptake sites. The iodinated neuroprobe is of the formula:



wherein R can be a C_nH_{2n+1} group, where $n=0-6$, an alkenyl group, a monofluoroalkyl group including nF where $n=18$ or 19 , or a $^mC_nH_{2n+1}$ group where $n=1-6$ and where $m=11$ or 14 for at least one mC . Also R' can be a C_nH_{2n+1} group where $n=0-6$, a *p*-iodophenylmethyl group, a *p*-iodophenylethyl group, a phenylmethyl group, or a phenylethyl group. X can be an isotope of F, an isotope of Cl, an isotope of Br, an isotope of I, CH_3 , or $Sn(R''_1R''_2R''_3)$. R''_1 can be a C_nH_{2n+1} group where $n=1-6$, or an aryl group. R''_2 can be a C_nH_{2n+1} group where $n=1-6$, or an aryl group. R''_3 can be a C_nH_{2n+1} group where $n=1-6$, or an aryl group. Y can be H only if X is an isotope of I, or R' is a *p*-iodophenylmethyl group, or R' is a *p*-iodophenylethyl group. Otherwise Y must be an isotope of I. Also provided is a diastereomer of this embodiment wherein the carboxyl- R' group is in the alpha position.

In a further embodiment, the iodinated neuroprobe for mapping monoamine reuptake sites of the invention is of the formula:



PS 11/14 wherein R can be a C_nH_{2n+1} group where $n=0-6$, an alkenyl group, a
HB 2 monofluoroalkyl group including ^{18}F where $n=18$ or 19 , or a $^{14}C_nH_{2n+1}$
B14401 group where $n=1-6$ and where $m=11$ or 14 for at least one ^{14}C . R' can
HB 4 be a C_nH_{2n+1} group where $n=0-6$, a *p*-iodophenylmethyl group, a
5 *p*-iodophenylethyl group, a phenylmethyl group, or a phenylethyl
6 group. X can be an isotope of F, an isotope of Cl, an isotope
H 7 of Br, an isotope of I, CH_3 , or $Sn(R''_1R''_2R''_3)$. R''_1 can be a C_nH_{2n+1}
8 group where $n=1-6$, or an aryl group. R''_2 can be a C_nH_{2n+1} group where
B144 9 $n=1-6$, or an aryl group. R''_3 can be a C_nH_{2n+1} group where $n=1-6$, or
40 10 an aryl group. Y can be H only if X is an isotope of I, or R' is
L 11 a *p*-iodophenylmethyl group, or R' is a *p*-iodophenylethyl group.
12 Otherwise, Y must be an isotope of I. Further, W can be O, S,
HB 13 $(CH_2)_n$, $O(CH_2)_n$ where $n=1-6$, wherein X resides on a benzene ring of
14 the formula at an ortho, meta, or para position with respect to W,
15 and Y resides at any remaining position on the benzene ring. Also
16 provided is a further embodiment which is a diastereomer of this
40 17 embodiment wherein the carboxyl-R' group is in the alpha position.

18 For each of the foregoing embodiments there is provided a
19 precursor of the radiolabeled neuroprobe that lacks a radiotracer
20 atom, and a kit for preparing an associated iodinated neuroprobe.

21 Both the radiostable and radioactive variants of the iodinated
22 neuroprobe of the invention are useful for human and non-human
23 research. For example, in vivo and in vitro experiments can be
24 performed using the compounds of the invention to study dopamine
25 reuptake sites generally, and cocaine binding sites in particular.

DECL 1
P 2

DESCRIPTION OF THE DRAWING

3 The invention will be more fully understood from the following
4 detailed description, in conjunction with the accompanying figures
5 in which:

6 Fig. 1 shows prior art compounds compared to compounds of the
7 invention;

8 Fig. 2 shows regional activity in a baboon brain following
9 injection of a compound of the invention;

10 Fig. 3 shows a synthesis route for a compound of the
11 invention;

12 Fig. 4 shows regional areas of brain uptake of a compound of
13 the invention;

14 Fig. 5A shows regional activity in a baboon brain following
15 injection of compound of the invention; and

16 Fig. 5B shows regional activity in a baboon brain following
injection of a compound of the invention.

DECL 17
PG 18
B62 19
L 20
H62 22

DETAILED DESCRIPTION OF THE INVENTION

21 Metabolically stable cocaine analogs such as 2 β -carbomethoxy-
22 3 β -(4-iodophenyl)-tropene), an iodine-containing analog of β -CIT
23 (also designated RTI-55), as shown in Fig. 1, compound 3, have high
24 affinities for dopamine and serotonin reuptake sites in brain. As
will be discussed below, [^{123}I]- β -CIT is shown to be a SPECT (single
photon emission computed tomography) radiotracer for dopamine and
serotonin reuptake sites.

9

462 1 [123I]- β -CIT was prepared by reaction of the corresponding
L 2 tributyltin precursor with no-carrier added Na[123I] in the presence
B 3 of peracetic acid, followed by preparative HPLC on a C-18 column
B 4 with methanol/water/triethylamine (75/25/0.2) at a flow rate of 1.0
L 5 ml/min. The final product was formulated in 6 ml sterile saline
B14 6 containing 5-10% ethanol.

7 Six SPECT experiments were performed in four female baboons
B 8 (10 kg *Papio anubis*) under isoflurane anesthesia. The animals were
B35629/ 9 injected with 10.6 ± 1.4 mCi[123I]- β -CIT and scanned for 333 ± 25 min
L 10 in either the 810X Brain Imager (Strichman Medical Equipment; five
11 experiments) or the ASPECT device (Digital Sintiographics,
12 Cambridge, MA; one experiment), with these and subsequent data
35629/ 13 expressed as means \pm S.E.M. Serial 2-6 min images were
14 reconstructed assuming uniform attenuation equal to that of water
15 in an ellipse drawn around the brain. Data were decay-corrected to
16 the time of injection.

17 The highest activities were found in the striatal region and
TAI 18 reached peak levels at 179 ± 9 min (n=6) post injection
B35 19 (p.i.) (Fig. 2). Striatal activity was monitored in two animals for
B 20 an additional 190 and 260 min post peak values. In one animal,
21 striatal activity was virtually unchanged for the remaining 190 min
22 of the experiment. With reference to Fig. 2, in the second animal,
23 washout of striatal activity was fit to an exponential function and
H176 B3 24 had $T_{1/2} = 27$ h ($r = 0.92$).

25 The brain region which approximately overlay the mesencephalon
26 or midbrain area had the second highest levels of activity.

1 Midbrain values peaked earlier (45 ± 16 min p.i.; $n=6$) and washed out
H178B35 more rapidly ($T_{1/2} = 294 \pm 59$ min; $r=0.98 \pm 0.01$; $n=3$) than that in the
3 striatum.

4 At the time of peak striatal uptake, the ratios of regional
B25 5 brain activities were: striatum (100%); hypothalamus ($38.1 \pm 5.2\%$);
6 occipital lobe ($13.5 \pm 0.8\%$); temporo-parietal lobes ($14.3 \pm 2.0\%$);
7 frontal lobe ($10.3 \pm 1.0\%$); and cerebellum ($10.0 \pm 1.5\%$), all measured
8 with $n=6$.

31 9 (-)Cocaine (Fig. 1, compound 1) and CFT (Fig. 1, compound 2),
10 both potent dopamine and serotonin reuptake inhibitors, induced
11 rapid and dose-dependant displacement of both striatal and midbrain
31B12 activity. (-)Cocaine ($2.9 \mu\text{mol/kg}$) administered at 200 min p.i.
13 caused displacement of 17% of striatal and 49% of midbrain levels
14B2 within 30-65 min. At $14.7 \mu\text{mol/kg}$ administered at 230 min p.i.,
B15 the corresponding cumulative displacements were 62% and 77%,
16 respectively, within the same period of time.

15B2 17 CFT ($0.4 \mu\text{mol/kg}$) administered i.v. at 180 min p.i. caused
B18 displacement of 57% of striatal and 72% of midbrain levels within
14B92 18 60-120 min. At $2.0 \mu\text{mol/kg}$ administered at 298 min p.i., the
B20 corresponding cumulative displacements were 83% and 91%,
21 respectively, within the same period of time.

22 In contrast, citalopram (a selective serotonin reuptake
23 inhibitor) caused greater displacement of midbrain than striatal
B824 activity. At a dose of $8.3 \mu\text{mol/kg}$ i.v. at 190 min p.i., midbrain
L25 levels decreased by 57% during the following 110 min, compared to
B26 only 5% decrease in striatal activity during the same period.

4621 [123I]- β -CIT appears to be a useful SPECT tracer of the dopamine
2 and serotonin reuptake sites. Brain uptake and washout are
3 relatively slow in comparison to cocaine itself and are consistent
624 with the metabolically resistant chemical structure of β -CIT and
5 the location of the radioiodine in a chemically stable position.
6 Striatal uptake appears to largely represent labeling of the
7 dopamine reuptake site, whereas that in the midbrain is largely
8 associated with the serotonin reuptake site. The high ratios of
H629 striatal to cerebellar activity of [123I]- β -CIT are consistent with
LL10 low non-specific uptake of the tracer, and suggest that [123I]- β -CIT
11 may be a useful clinical marker of dopaminergic deficiencies in
12 Parkinson's disease.

13 Referring again to Fig. 1, in a second study (Neumeyer, J.L.
B144 et al., J. Med. Chem., 34: 3144-3146, 1991), the potent cocaine
L625 analog 2 β -carbomethoxy-3 β -(4-fluorophenyl)tropane (compound 2)
B16 (also referred to as CFT or WIN 35,428 (Clarke, R.L., et al., 1973;
LH17 Madras, B.K. et al., 1989)) when tritiated or labeled with ¹¹CH₃ was
H18 found to be superior to [³H]cocaine or [¹¹C]cocaine (Fowler, J.S. et
B1419 al., Synapse 4: 371-377, 1989) as a radioligand probe for cocaine
20 receptors in terms of higher affinity and larger residence time on
21 the dopamine reuptake site. For further development of analogues
B622 suitable for PET and SPECT imaging, 2 β -carbomethoxy-3 β -(4-
23I iodophenyl)tropane were synthesized and characterized (compound 3a;
6224 designated as β -CIT in analogy to CFT, its corresponding, N-
25 demethylated derivative (compound 4; designated as nor-CIT), and
H126 the C_{2 α} isomer (compound 3b), as shown in Fig. 1.

H621 Referring to Fig. 3, a synthesis protocol for [^{123}I]- β -CIT is
2 described. Ecgonidine methyl ester (compound 5) was prepared from
B 3 cocaine by the procedure of Clarke et al. (1973.) Treatment of
4 compound 5 with phenylmagnesium bromide and subsequent workup with
H 5 trifluoroacetic acid at low temperature gave a mixture of C_2 epimers
B 6 (compound 6) (45%) and (compound 7) (31%), which were separated by
H B flash chromatography (silica; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, 25:1). Direct iodination
H 8 of compound 6 with $\text{I}_2/\text{HNO}_3/\text{H}_2\text{SO}_4$ gave the para-substituted compound
I 6 3a (β -CIT) as an oil; 62%; $[\alpha]_{\text{D}}^{25} -2.0^\circ$ ($c = 0.85$, CHCl_3). D-Tartrate
B 4 101 salt; mp $72-74^\circ\text{C}$; $[\alpha]_{\text{D}}^{25} -87.7^\circ$ ($c = 1.5$, CH_3OH). Iodination of
I 6 11 compound 7 by the same procedure gave compound 3b (α -CIT) as an
B 6 12 oil; 39% $[\alpha]_{\text{D}}^{25} +44^\circ$ ($c = 2.5$, CHCl_3). 1,5-naphthalenedisulfonate
B 4 13 salt; mp $139-140^\circ\text{C}$. N-Demethylation of compound 6 was
B 14 accomplished by conversion to its 2,2,2,-trichloroethyl carbamate
15 followed by reduction ($\text{Zn}/\text{acetic acid}$) to yield compound 8 by the
16 procedure previously described by Milius, R.A., et al., J. Med.
B 14 17 Chem. Vol. 34, No. 5, 1728-1731, 1991, herein incorporated by
18 reference, followed by iodination to yield nor-CIT (compound 4),
B 19 which was isolated as a yellow crystalline solid (free base 48%
B 14 160 31 from compound 6): mp $149-151^\circ\text{C}$; $[\alpha]_{\text{D}}^{25} -67.4^\circ$ ($c = 1$, CHCl_3).

H62 21 [^{123}I]- β -CIT (compound ^{123}I -3a) was synthesized from
62 22 nonradioactive β -CIT (compound 3a) by conversion to the
23 corresponding tributyltin derivative (compound 9). Treatment of
J 24 compound 3a with bis(tributyltin),
25 tetrakis(triphenylphosphate)palladium(0), and palladium(II) acetate
26 in refluxing tetrahydrofuran gave compound 9 as a colorless waxy

solid after flash chromatography (silica, stepwise gradient, hexane to hexane/ether, 75:25) in 26% yield from 3a. The 300-MHz NMR (CDCl₃) of compound 9 was consistent with the assigned structure. Reaction of compound 9 with no-carrier-added Na¹²³I in the presence of peracetic acid gave compound [¹²³I]-3a. The radioiodinated product compound [¹²³I]-3a was purified by preparative HPLC (Novapak C₁₈, MeOH/H₂O/Et₃N, 75:25:0.2, 1.0 mL/min; t_R 6.7 min) and formulated in normal saline containing 5% ethanol and 1% ascorbic acid. Compound [¹²³I]-3a was obtained in average overall yield of 60.0 ± 13.4% and with radiochemical purity of 97.6 ± 1.6%. The tributyltin precursor used in radiolabeling contained about 7 mol% CIT carrier, resulting in an ¹²³I product having a specific activity of about 2000 ci/mmol.

The affinities of cocaine (compound 1), α-CIT (compound 3b), β-CIT (compound 3a), and β-CFT (compound 2) for the dopamine and serotonin reuptake sites were determined from radioligand displacement studies using tissue homogenates prepared from baboon and rat brain, shown in Table 1 below.

Table I. In Vitro Radioligand Binding Data for Cocaine and 3-(4-Halophenyl) Analogues^a

analogue	displacement of [³ H]CFT		displacement of [³ H]paroxetine	
	IC ₅₀ (nM)	Hill slope (nH)	IC ₅₀ (nM)	Hill slope (nH)
1 (cocaine)	221 ± 14	0.69 ± 0.06 (3)	207 ± 66	0.73 ± 0.12 (5)
2 (β-CFT)	15.3 ± 1.2	0.75 ± 0.01 (3)	479 ± 59	1.34 ± 0.22 (3)
3b (α-CIT)	87.6 ± 2.9	0.70 ± 0.07 (2)	210 ± 86	0.73 ± 0.04 (2)
3a (β-CIT)	1.6 ± 0.15	0.79 ± 0.04 (3)	3.78 ± 0.53	0.82 ± 0.08 (6)

TABLE 1

PBH
B 2 The data in Table 1 represent radioligand binding of [³H]CFT
H 3 (0.5 nM) to dopamine reuptake sites in tissue homogenates prepared
4 from primate striatum and binding of [³H]paroxetine to serotonin
H 5 reuptake sites in homogenates prepared from rat cortical membranes.
B 6 The IC₅₀ value is the concentration of displacing analogue required
35 7 to decrease specific radioligand binding by 50%. Values represent
means ±SEM (of n experiments).

8 With reference to Fig. 4, five SPECT (single photon emission
9 computer tomography) experiments were performed with four female
B 10 baboons (*Papio anubis*, 10-12 kg) under isoflurane anesthesia.
B 35 11 Animals were injected i.v. with 8.1±1.4 mCi [¹²³I]-β-CIT (with these
35 12 and subsequent data expressed as mean ± SEM) and scanned for 300±41
B 13 min with the 810X Brain Imager (Strichman Medical Equipment,
B 14 Medfield, MA). Serial 1-2 min images were reconstructed assuming
15 uniform attenuation equal to that of water in an ellipse drawn
16 around the brain. Data were decay corrected to time of injection.

20 17 Highest brain uptake overlay the striatal region and peaked at
A 2 18 154 ± 19 min postinjection (pi) of the radioligand and showed
B 35 19 striatal to cerebellar ratios at that time of 9.8 ± 1.6. Washout
LL 20 of striatal activity was followed for an additional 200 and 260 min
B 21 in two of three control animals and showed 0% and 12 % decreases,
L 22 respectively, from time of striatal peak to end of the experiment.

23 With reference to Figs. 5A and 5B, the brain area with second
24 highest activities approximately overlays the midbrain and showed
B 35 25 peak levels at 43±5 min pi (n=5) and had a faster washout than
26 striatal activity.

624
B3
68.3
66
6
B
L
682
B13
B14
L35
16
H627

The pharmacological specificity of the in vivo labeling of [¹²³I]-β-CIT was examined with displacement of brain activity by indatraline (also designated Lu 19-005), a potent agent for the dopamine and serotonin reuptake sites, and citalopram, an agent selective for the serotonin reuptake site. Indatraline (3 μmol/kg iv) injected at 200 min pi radioligand caused significant decrease of both striatal and midbrain activity, as shown in Fig. 5A. During the 100 min period after injection of Lu 19-005, striatal activity decreased by 65% compared to a mean decrease of 2% during the same period in the two control animals followed for that length of time. In contrast, citalopram (7.4 μmol/kg iv) injected 60 min pi radioligand showed a selective decrease of midbrain activity, as shown in Fig. 5B. Citalopram caused a 48% decrease of midbrain activity during the 60-min period after injection, in comparison to 16 ± 3% decrease (n=3) of midbrain activity in control animals followed during this same period.

H627
624

These results showed that [¹²³I]-β-CIT was a useful SPECT probe of monoamine reuptake sites in primates. The majority of striatal activity was associated with dopamine reuptake sites, and the majority of midbrain activity was associated with serotonin reuptake sites, which is consistent with the densities of these monoamine transporters measured in postmortem primate brains. Brain washout of activity was relatively slow, in part because of the high affinities of β-CIT for the monoamine transporters. In addition, the iodine atom appears to be in a relatively metabolically resistant position, since whole body scanning showed

low thyroid uptake, which is indicative of a slow in vivo rate of
deiodination. [¹²³I]-β-CIT and [¹¹C]-β-CIT may be useful clinical
markers of dopaminergic and serotonergic innervation in human
disorders such as Parkinson's disease and depression, which are
thought to have abnormalities in these neuro-transmitter systems.

EXAMPLES OF SYNTHESSES

Example 1. 2-beta-Carbomethoxy-3-beta-(4-iodophenyl)tropane

A mixture of 2-beta-carbomethoxy-3-beta-phenyltropane (See
Example 1A below and Milius et al. J. Med. Chem., 1991, 34, 1728)
(2.9g, 11.5 mmol) and I₂ (3g. 11.8 mmol) in 25 ml of glacial acetic
acid was stirred and treated dropwise with a mixture of 4.7 mL of
concentrated nitric acid and 4.7 mL of concentrated sulfuric acid.
The reaction mixture was heated to 55°C and stirred for 2 hours,
then cooled to room temperature and poured onto ice (100g) and
filtered. The pH of the filtrate was adjusted to 9.5 by the
addition of concentrated ammonium hydroxide at 0-5°C. The
resulting precipitate was removed by filtration and dissolved in
methylene chloride (250ml). The filtrate was extracted with two 50
mL portions of methylene chloride. The extracts and solution of
precipitate were combined, washed with brine (50ml) and dried over
magnesium sulfate. After the removal of the solvent, 3.9 g (90.4%)
of 2-beta-carbomethoxy-3-beta-4-iodophenyltropane free base was
obtained as an oil.

The free base was dissolved in methanol (20 ml) and combined
with 1.5 g of D-(-)tartaric acid in 20 ml of methanol. After the

1 removal of methanol under reduced pressure, the residue was
2 recrystallized from methanol ether (3:1) to give 2-beta-
3 carbomethoxy-3-beta-(4-iodophenyl)tropane D-tartrate salt as white
4 crystals, m.p. 72-74°C. $C_{16}H_{20}NO_2I \cdot C_4H_6O_6$. Calculated: C: 44.88, H:
5 4.89, N: 2.62. Found: C: 44.70, H: 4.94, N: 2.57. $[\alpha]_D^{22} =$
6 $-87.7^\circ (c=0.3, CH_3OH)$.

Example 1A. 2-beta-Carbomethoxy-3-beta-phenyltropane

8 A 2 M ethereal solution of phenylmagnesium bromide (83 mL, 166
9 mmol) in a 500-mL 3-neck round-bottom flask equipped with
10 mechanical stirrer, addition funnel, and nitrogen inlet tube was
11 diluted with 83 mL of anhydrous diethyl ether and cooled to $-20^\circ C$
12 under an atmosphere of dry nitrogen. A solution of anhydroecgonine
13 methyl ester, prepared from cocaine (1) (15 g, 82.8 mmol) in
14 anhydrous ether (75 mL) was added dropwise. The heterogeneous
15 mixture was stirred for 1 h at $-20^\circ C$, then poured into an equal
16 volume of ice and water, and acidified by the dropwise addition of
17 2 M HCl. The aqueous layer was made basic by the addition of
18 concentrated ammonium hydroxide, saturated with NaCl, and extracted
19 with diethyl ether. The combined extracts were dried (Na_2SO_4) and
20 concentrated in vacuo to give a brown oil. Bulb to bulb
21 distillation ($70^\circ C$, 0.9 Torr) of the crude product gave a pale
22 yellow oil (16 g, 70%). TLC analysis of the oil (silica,
23 pentane/diethyl ether/2-propylamine, 15:5:0.8) showed it to be a
24 mixture of the C-2 alpha and beta epimers. The beta isomer was
25 isolated by silica gel chromatography (pentane: diethyl ether:

B14
L 1 isopropyl amine, 70:30:3). m.p. 63-66°C (lit: 62-64,5°C: Clarke et
2 al. J. Med. Chem.. 16: 1260 (1973)).

Clarke
PB 4 Example 2. 2-alpha-Carbomethoxy-3-beta-iodophenyltropane

5 The mixture of alpha and beta-2-carbomethoxy-3-beta-
6 iodophenyltropanes prepared as described in Example 1 were
7 separated by silica gel chromatography as described in Example 1.
8 Fractions containing the alpha-2-carbomethoxy-3-beta-
9 iodophenyltropane were pooled and concentrated in vacuo. The free
10 base thus obtained was treated with naphthalene-1,5-disulfonic
11 acid. The crude salt was recrystallized from acetonitrile to give
12 the 2-alpha-carbomethoxy-3-beta-iodophenyltropane naphthalene-1,5-
13 disulfonate salt, m.p. 166-168°C. $C_{16}H_{20}NO_2I \cdot C_{10}H_6(SO_3H)_2 \cdot 2H_2O$.
14 Calculated: C:40.01, H:4.55, N:1.97, I:17.90; Found: C:43.94,
H:4.55, N:1.91, I:17.99.

Clarke
PB 6 Example 3. 2-beta-Carbomethoxy-3-beta-(4-iodophenyl)nortropine.

16 A solution of 2-beta-carbomethoxy-3-beta-(4-iodophenyl)tropane
17 (410 mg, 1.5 mmol) in toluene (20 mL) was treated with of 2,2,2-
18 trichloroethyl chloroformate (1 mL, 7.3 mmol). The mixture was
19 heated at 120°C for 1 hour, cooled to room temperature, and
20 evaporated to dryness in vacuo. The residue was partitioned
21 between methylene chloride and water. The organic layer was
22 separated, dried (Na_2SO_4), and concentrated in vacuo to give the
23 trichloroethyl chloroformate as a dry foam. The crude carbamate
24 was dissolved in 50% aqueous acetic acid, treated with 200 mg

B
L
H
B
B/HH
1 (0.0067 g-atom) of zinc dust, and stirred at room temperature for
2 16 hours. The reaction mixture was filtered adjusted to pH 7 with
3 concentrated ammonium hydroxide, saturated with NaCl, and extracted
4 with diethyl ether. The extracts were combined, dried (Na₂SO₄), and
5 concentrated in vacuo. The residue was purified by flash
6 chromatography (silica, pentane/diethyl ether/isopropylamine,
7 3:7:0.7) to afford 2-beta-carbomethoxy-3-beta-(4-iodophenyl)nortropane, which was isolated as a yellow crystalline
8 solid, m.p. 149-151°C; [alpha]_D²⁵ -67.4° (c = 1, CHCl₃).

Example 4. 2-beta-Carbomethoxy-3-beta-(4-iodophenyl)-8-(3-fluoropropyl)-nortropane

B
PB
H
B
12 A solution of 2-beta-carbomethoxy-3-beta-(4-iodophenyl)-
13 nortropane (371 mg, 1.0 mmol), 1-bromo-3-fluoropropane (155 mg,
14 1.1 mmol), and triethylamine (0.5 mL) in dry toluene (20 mL) was
15 stirred under an atmosphere of dry nitrogen and heated to reflux.
16 After four hours, the reaction mixture was cooled to room
17 temperature and filtered. The filtrate was concentrated under
18 reduced pressure, and the residue chromatographed on a silica
19 column (eluant: diethyl ether). Concentration of product-
20 containing fractions gave 2-beta-carbomethoxy-3-beta-(4-iodophenyl)-8-(3-fluoropropyl)nortropane as a white solid, m.p.
21 78.5-79.5°C C₁₈H₂₃NO₂FI, Calculated: C: 50.13, H: 5.34, N: 3.25; Found:
22 C: 50.27, H: 5.26, N: 3.15.
23

CLUB 21 B
2
Example 5. 2-beta-Carbomethoxy-3-beta-(3-fluoro-4-iodophenyl)tropane

PB 3 A mixture of 2-beta-carbomethoxy-3-beta-(3-fluorophenyl)
4 tropane (400 mg, 1.44 mmol), silver sulfate (400 mg, 1.3 mmol),
5 iodine (600mg, 2.36 mmol) and 80% sulfuric acid (9 Ml) was stirred
6 for five days at room temperature. The reaction mixture was poured
B 7 into 150 mL of ice and water, made basic by the addition of
L 8 concentrated ammonium hydroxide, and extracted with three 60 mL
9 portions of chloroform. The combined extracts were washed
B 10 sequentially with solutions of 10% sodium bisulfite, 5% sodium
11 carbonate and water, then dried over sodium sulfate, and filtered.
12 The filtrate was concentrated in vacuo and the oily residue was
13 redissolved in chloroform and treated with a solution of p-toluene
14 sulfonyl chloride in chloroform. The resulting solid was
B 15 repeatedly recrystallized from water and ethanol to give 2-beta-
16 carbomethoxy-3-beta-(3-fluoro-4-iodophenyl)tropane tosylate salt as
17 a white crystalline solid, m.p. 68-70°C (soften, 45°C), $C_{16}H_{19}FINO_2$
18 • $C_7H_8SO_3$ • H_2O : Calculated: C: 46.55, H: 4.93, N: 2.36; Found: C:
19 46.34, H: 4.86, N: 1.99.

CLUB 21 B
PB 21
L 22
Example 6. 2-beta-Carboxy-3-beta-(4-iodophenyl)tropane

21 A suspension of 2-beta-carbomethoxy-3-beta-(4-
22 iodophenyl)tropane (100 mg, 0.26 mmol) in 2 mL of H_2O was heated at
23 reflux for 10 hours. The resulting solution was cooled to room
24 temperature, and the resulting precipitate was collected by
B 25 filtration and dried under vacuum overnight to give 70 mg (70%) of

B14 1 2-beta-carboxy-3-beta-(4-iodophenyl)tropane m.p. 299-300°C.
H B 2 C₁₅H₁₈NO₂I . 0.5 H₂O: Calculated C: 47.51, H:5.05, N: 3.69: Found:
L 3 C: 47.28, H: 4.84, N: 3.69.

Chubb^g 4 CB
PB 5 A
L 6
B 7
B 8
B 9
L 10
11
B 12
L 13
14
B 15
16
B 17
L 18
B 19
L 20
L 21
22
B 23
L 24
L 25
22

Example 7. 2-beta-Carbomethoxy-3-beta-benzyloxytropane

A stirred suspension of benzyl bromide (3.0 g, 0.015 mol) and potassium iodide (3.0 g, 0.021 mol) in acetone (20 mL) was treated dropwise with a solution of ecgonine methyl ester (2.6 g, 0.014 mol) in acetone (10 mL) at room temperature. The mixture was stirred at room temperature for 70 hours, then heated to reflux and stirred for an additional 8 hours. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo, the residue dissolved in chloroform (200 mL) and extracted with four 50 mL portions of 2 N hydrochloric acid. The combined extracts were made basic by the addition of concentrated ammonium hydroxide. The resulting mixture was extracted with four 20 mL portions of chloroform. The extracts were dried over sodium sulfate and concentrated in vacuo to give 1.7 g of 2-beta-carbomethoxy-3-beta-benzyloxytropane as an oil.

The product was dissolved in acetonitrile (20 mL) and treated with a solution of naphthalene-1,5-disulfonic acid (2.2 g) in acetonitrile (20 mL). The solution was concentrated in vacuo to a syrup, which was diluted with diethyl ether. The resulting precipitate was collected by filtration and dried to give 1.6 g of 2-beta-carbomethoxy-3-beta-benzyloxytropane naphthalene-1,5-disulfonate salt, m.p. 126-130°C, C₁₇H₂₃NO₃.C₁₀H₆(SO₃H)₂.2.5 H₂O.

1 Elemental analysis: Calculated, C: 52.08, H: 5.83, N: 2.25. Found,
C: 52.02, H: 5.69, N: 2.72. $[\alpha]_D^{24} = -25.4^\circ (c=1, \text{CH}_3\text{OH})$.

Example 8. 2-beta-Carbomethoxy-3-beta-(4-tributylstannylphenyl)tropane

A mixture of 2-beta-carbomethoxy-3-beta-(4-iodophenyl)tropane (250 mg, 0.65 mmol), bis(tributyl)distanane (522 mg, 0.9 mmol), tetrakis(triphenylphosphine)palladium(0) (3 mg) and anhydrous toluene (10 mL) was heated to reflux under an atmosphere of dry nitrogen and stirred for 28 hours. The mixture was filtered, and the filtrate concentrated in vacuo. The residue was applied to a silica gel column and eluted with a mixture of hexane : diethyl ether : isopropyl amine (70:30:3). The fractions containing product were pooled, concentrated in vacuo and treated with pentane to precipitate 2-beta-carbomethoxy-3-beta-(4-tributylstannylphenyl)tropane as a solid. The 300 MHz NMR spectrum was consistent with the assigned structure. $[\alpha]_D^{22} = -8.9^\circ (c=0.4, \text{CHCl}_3)$.

Example 9. ^{123}I -2-beta-Carbomethoxy-3-beta-(4-iodophenyl)tropane

To a vial containing 50 μg (0.094 μmol) of 2-beta-carbomethoxy-3-beta-(4-tributylstannylphenyl)tropane was added 50 μL ethanol, 150 μL 0.5M H_3PO_4 , 125-500 μL (20-30 mCi) ^{123}I NaI solution, and 100 μL (4.2 μmol) 0.042M peracetic acid. After 20-30 minutes, 50 μL of 100mg/mL aqueous NaHSO_3 solution was added. Saturated NaHCO_3 solution was added, and the mixture extracted with

H 1 ethyl acetate. The combined extracts were dried (Na_2SO_4) and
2 concentrated to dryness. The residue was redissolved in methanol
B 3 and purified by HPLC (C-18 column, eluant: CH_3OH : H_2O :
L 4 triethylamine; 75:25:0.2). The fraction eluting at the retention
B 5 time of 2-beta-carbomethoxy-3-beta-(4-iodophenyl)tropane was
B 6 collected evaporated to dryness and reconstituted in 5% ethanol and
B 7 0.1 nM ascorbic acid.

8 In SPECT applications, the radiostable iodinated neuroprobe of
9 the invention is useful as a reference standard, and can also be
10 used as a dilutant for the radioactive form of the neuroprobe. The
11 radioiodinated compound is generally identified by its
12 chromatographic mobility as compared with a fully characterized
13 reference standard. Thus, preparation of the radioiodinated
14 compound requires the non-radioactive iodinated compound.

15 To avoid the necessity of storing a radioactive neuroprobe, it
16 is useful to provide a kit containing the non-radioactive iodinated
17 compound and an appropriate oxidizing agent, such as perchloric
18 acid, performic acid, peracetic acid, hydrogen peroxide, hydrogen
B 19 peroxide with lactoperoxidase, 1,3,4,6-tetrachloro-3 α ,6 α -
L 20 diphenylglycouril, or a N-chloro-4-methylbenzenesulfonamide sodium
21 salt. Then, the non-radioactive precursor compound can be oxidized
22 in the presence of a suitable radioactive compound, such as the
23 carrier free $\text{Na}[^{123}\text{I}]$ shown in the synthesis route described herein,
24 any other radioisotope source, such as any solution of a salt of a
H 25 radioactive isotope of iodine, a reagent containing $^m\text{C}_n\text{H}_{2n+1}\text{X}$, where
B 26 n=0-6 and X is a leaving group, or a reagent containing ^{18}F of the

AB141 formula $FC_nH_{2n}X$, where $n=0-6$ and X is a leaving group, to prepare the
2 iodinated neuroprobe at its time and place of use.

H 3 Radiolabeled neuroprobes of the invention are also useful in
4 other imaging procedures. For example, an ^{125}I -labeled neuroprobe can
5 be used in autoradiography or therapy, and an ^{131}I -labeled neuroprobe
6 is useful as a multiple photon emitter for use in animal studies.
H 7 Also, ^{11}C -, ^{14}C -, and ^{18}F -labeled neuroprobes can be used in PET
8 imaging.

9 Both the radiostable and radioactive variants of the iodinated
10 neuroprobe of the invention are useful for human and non-human
11 research. For example, in vivo and in vitro experiments can be
12 performed using the compounds of the invention to study the
13 dopamine transporter generally, and cocaine binding sites in
14 particular.

15 Additionally, the radiostable version of the neuroprobe of the
16 invention can be used as a drug for influencing dopamine reuptake.

17 Other modifications and implementations will occur to those
18 skilled in the art without departing from the spirit and the scope
19 of the invention as claimed. Accordingly, the above-description is
20 not intended to limit the invention except as indicated in the
21 following claims.